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Bacteriocins: Isolation and Characterization from Bacteria Inhabiting Different Habitats

DEPARTMENT OF BIOTECHNOLOGY

FACULTY OF ENGINEERING & TECHNOLOGY

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ABSTRACT

Bacteriocins are ribosomally synthesized antimicrobial peptides produced by microorganisms belonging to different eubacterial taxonomic branches. Most of them are small cationic membrane-active compounds that form pores in the target cells, disrupting membrane potentials and causing cell death. Although the structure, function and biosynthesis of many bacteriocins are known, many aspects of these compounds are still unknown. Most of the bacteriocins identified till now are having the narrow spectrum antimicrobial activity i.e. they can be effective against very few species. Now a days, the research on bacteriocin is focused for its broad spectrum antibacterial activity with the objective that bacteriocin can handle a number of species. The proposed research work is planned to purify and characterize the broad spectrum bacteriocin from specific microbial habitats/sources i.e. soil, polluted water, industrial waste, food waste and sewage.This intiative will contribute towards use of these bacteriocin as an effective alternative to antibiotics and could be considered as an alternative treatment of various diseases without any side effect.

Key Words : Bacteriocins, Microbial habitats, Broad spectrum antimicrobial activity, Alternative treatment.

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1. INTRODUCTION

From the time of Louis Pasteur & Robert Koch scientists have recognized that micro organisms cause infectious diseases and need to be controlled and eliminated from our surroundings. Penicillin discovered by Alexander Fleming in 1929 unlocked the door for the use of antimicrobials for both humans and animals to eliminate pathogens. Bacteriocin are alike antibiotics, when it comes to combat harmful microorganisms and their use has come into prominence lately. Bacteriocins are produced by Gram-negative as well as Gram positive bacteria. These are amino acid based compounds with antimicrobial properties which form a heterologous class of ribosomal synthesized antibacterial peptides **(De vuyst & Vandamme 1994 ; Line et al. 2008)**. These are proteinaceous toxins released by bacteria to inhibit the growth of other bacteria, mostly closely related to the bacteriocin producing strain. These toxins are quite similar to those produced by yeast and paramecium **(Cotter et al. 2012)**.

Figure 1 : Bacteriocin producing bacteria (*Lactococcus lactis*)

A Gram-positive bacterium, *Lactococcus lactis*, first reported by Rogers in 1928 is the first genetically modified organism, to be used alive for the treatment of human disease **(Madigan & Martinique 2005)**. Food and Drug Administration (FDA) approved for the use of Lactobacillus and their products, which would include bacteriocins, as GRAS i.e. Generally recognized as safe **(Cleveland et al. 2001)**. Some of the important genera having bacteriocin producing capability are *Lactococcus, Lactobacillus, Enterococcus, Carnobacterium* and *Streptococcus.* Bacteriocins from Gram-negative microorganisms emerge predominantly from Enterobacteriaceae. These bacteriocins are mostly small peptides with a molecular weight of 3-6 KDa **(Nes et al. 1996)**. Majority of Gram-positive bacteriocins are membrane active and mechanism of action includes increased penetrability of the cytoplasmic membrane **(Jack et al. 1995)**. They possess a large spectrum of bactericidal activity which might be due to the following reasons:

- Their mode and mechanism of action.
- They do not require a specific cell surface receptor.

- The absence of an outer bacterial membrane which would restrict their access to cytoplasmic membrane.

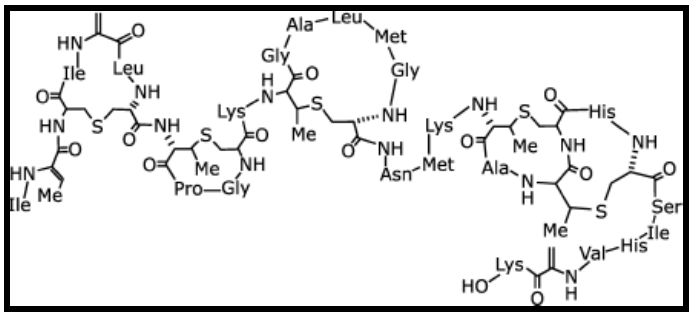

Figure 2 : Molecular structure of Bacteriocin (Nisin) **Figure 3 : Nisin as a food preservative**

Bacteriocins have been found to inhibit the growth of disease causing microbes. The parent microbes producing this peptide remain unaffected. These peptides mostly shows narrow spectrum of activity towards related micro-organisms. Bacteriocins producing bacteria have an advantage that they can survive and resist through a combative environment **(Bianchi et al. 1999)**. They stimulate the production of Interferon gamma and secretary IgA in response to foreign antigen and also play a role in transportation of these antigens to intestinal Peyer,s patches to eliminate the harmful antigens **(Herich & Levkut 2002)**. Their activity against disease causing micro-organisms has driven research into their possible role as therapeutic agents in recent years. There is need for today that scientists should find different ways to overcome the phenomenon of drug resistance and the deleterious side effects of antibiotics ingestion. Bacteriocins demonstrate properties that could provide an answer to both the above mentioned limitations and are thus evoking lot of interest among researchers **(Gillor et al. 2005)**.

There are various sources from which bacteriocin producing bacteria were isolated i.e. *L. reuteri*, *L. bavaricus* and *L. plantarum* were isolated from sour dough sample **(Ganzle 2004 ; Larsen et al. 1993)**, *B. lireus* from cheese sample **(Motta & Brandelli 2003)**, *E. faecium* from goat meat **(Dutta et al. 1999)**, *Pediococcus acidilactici* from Chilli pickle sample **(Kaur & Balgir 2004)**, *B. lentus* and *L. plantarum* from yoghurt **(Sharma et al. 2006 ; Aly & Abo 2007)** and from raw barley *lactobacillus* spp. was isolated **(Hartnett et al. 2002)**.

Now a days, bacteriocins are also having another application. They are broadly used in food industry for extending the duration of food preservation **(Ghrairi et al. 2012)** in meats, vegetables, dairy, and other different food products. They are known to inhibit the bacterial contamination while food production process progresses **(Galvez et al. 2008; Khan et al. 2010)**. The bacteriocin application is also included in improvement of safety and quality of the fermented food **(Mezaini et al. 2009 ; Jin & Zhang 2008)**. In meat industry , a bacteriocin (Enterocin) inhibit the growth of *L. monocytogenes* in sausage, pork, chicken breast like meat

products **(Aymerich et al. 2000a ; Aymerich et al. 2000b)**. Bacteriocins are also used to cure veterinary diseases **(van Heel et al. 2011)**, and in medical field for treatment of chronic cancers **(Lancaster et al. 2007)**.

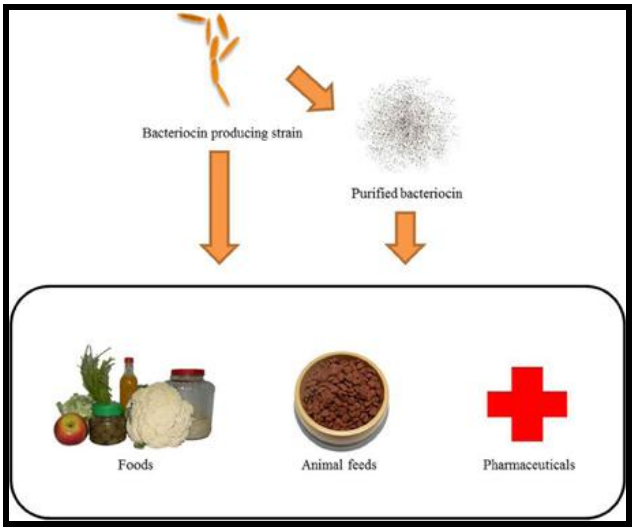

Figure 4 : Uses of bacteriocin

There are different mechanisms by which bacteriocins exert their action. They may act in combination with other compounds like lactoferrins, lysozymes etc. or they may accumulate in high concentrations at a particular spot as part of an inflammatory response. Another important way in which they exert their action is by acting as immune modulators **(Guani-Guerra et al. 2010)** by influencing host cell gene expression or as chemokine or play a role in stimulating chemokine production. Their action on dendritic cells activates the T cells and initiates the immune response **(Yuping & Richard 2009)**.

As considering the potentiality as broad spectrum activity and limitation as narrow spectrum activity of bacteriocin, they could be utilize as an alternative to antibiotics. The proposed research study plan is designed to isolate, purify and characterize the broad spectrum bacteriocin.

2. LITERATURE REVIEW

The nature of antibiotic compound produces by *E.coli*. Strain V (virulent in experimental infections) produces a dialyzable and heat-stable substance in liquid media (later referred to as colicin V) that inhibited the growth of *E. coli* in high dilution was first demonstrated and documented by **Gratia (1925)**. The term ‘‘bacteriocin’’ was coined by **Jacob et al. (1953)** to denote antibacterial proteins demonstrating narrow spectrum of activity against related species. **Fredericq (1957)** classified the microorganism according to the specificity of bacteriocin adsorption. The classification of bacteriocin is mainly based on colicins and later

classified into sub groups as per the specificity of their immunity and spectrum of resistance. As a result, sixteen classes of bacteriocin on the basis of species that produced them **(Revees 1965)** were classified. Based on this work, colicins are bacteriocins produced by E. coli; Pyocin produced by *Pseudomonas aeruginosa* (formely pyocinia). *Enterobacter cloaceae* produced a bacteriocin named Cloacin and so on. All this work has led to the establishment of a code of nomenclature as given by **Ohno et al. (1977)**. Colicin V is different from most of the colicins isolated later on because of its small size and stability at low temperature **(Faith et al. 1992)**.

According to the latest classification the bacteriocins are divided into three groups:

- **Class I:** This group is again subdivided into Type A and Type B lantibiotics as indicated by the compound structure and the antimicrobial exercises. Nisin, belongs to this gathering of bacteriocins.
- **Class II:** Small (< 10 k Da), temperature stable, non - lanthionine containing peptide are contained in this group.
- **Classes III:** It contains (> 10 K Da) heat labile proteins.

In early research, Fujimura and his research team collected swab from human dental plaque. From these samples *Streptococcus sanguis* strain N-2 was observed to produce a bacteriocin named **Sanguicin** and it was recognized as a bacteriostatic agent because it acted on susceptible cells **(Fujimura et al. 1979)**. The rate of production of bacteriocin was higher among human clinical isolates. This led to the recommendation that the study of disease transmission of vancomycin resistance may be impacted by diverse variables, including bacteriocin production **(Campo et al. 2001)**.

Pattnaik et al. (2001) characterized a bacteriocin-like factor (Lichenin) from *Bacillus licheniformis* that was isolated from rumen of buffalo. Lichenin exhibited the bactericidal activity against a number of bacterial strains. These could be a good agent for manipulating the rumen infection. **Pal et al. (2005)** found ten LAB isolates from food sample (South Indian Dosa) showing good antimicrobial activity against *Bacillus, Staphylococcus, Listeria, Pseudomonas, Vibrio* and *Aeromonas*. In this study characterization of isolates were done through morphological, physiological, biochemical and carbohydrate fermentation tests.

Jabeen et al. (2009) isolated *Argobacterium radiobacter* from agricultural soil sample that produced antibacterial peptide designated as Agrocin NA6. The Agrocin had a narrow spectrum of activity. It was heat stable and active in presence of different heavy metal salts and could be used as bio-control agent. The Bb bacteriocin from *Bacillus brevis* and pyocin Pa from *Pseudomonas aeruginosa* were isolated and found to be bioactive only against some gram-positive bacterial strains **(Saleem et al. 2009)**.

Sharma et al. (2011) purified and characterized a bacteriocin from *Bacillus subtilis* isolated from fermented chunks of mung bean sample. The Bacteriocin can act against many food spoilage pathogens. This was isolated and purified by molecular sieve chromatography. A new

bacteriocin - Pumiviticin from *Bacillus Pumillus* was isolated and characterized from sea water soil Sample (Endore, India). The Pumiviticin having a molecular weight of 3.9 KDa inhibits a wide range of microorganism belonging to the lactic acid bacteria group **(Dasarathan et al. 2012)**. Another potential role of bacteriocin identified in food applications, their numerous conceivable uses for the control of unwanted microorganisms in the human environment is incredibly overlooked.

3. DESCRIPTION OF BROAD AREA

The discovery and use of antibiotics is one of the most significant application of microbial flora. However, the quick ascent and spread of antibiotic-resistant bacterial pathogens has contributed to look alternatives of substitute routes for fighting diseases. One of the reasons, for utilizing the antibiotics is that they can be used against various types of bacterial species and not impervious to the particular medication. As a result, antibiotic destroys normal bacterial flora by attacking both the harmful and beneficial bacteria in the intestines and other body parts in humans. This leads to an another infection as normal microbial flora get destroyed. Over and misuse of antibiotics create resistance in both pathogen and commensal microbes **(Walker 2001)**. Bacteriocins are a good option as an alternative mode of treatment. They can be considered as 'designer drugs' which target particular pathogen and related species for recovery from infections **(Riley & Wertz 2002)** so in future bacteriocins can be utilized as novel restorative operators **(Fimland et al. 2002)**. Bacteriocins having a broad spectrum of antimicrobial activity can be used as future antimicrobial agents with an important role in medicinal sciences. They also exhibit no side effects. Furthermore, bacteriocins can be used in food industry for elimanating the spoilage bacteria and increasing the food storage shelf life.

4. OBJECTIVES OF RESEARCH

1. To isolate bacteria from different physical sources.
2. To screen bacterial isolates for the production of bacteriocin.
3. To identify the bacteriocin producing isolates.
4. To extract bacteriocin from the selected isolates and determine its activity.
5. To study the spectrum activity of bacteriocins against standard pathogenic bacterial strains.
6. To purify and characterize the extracted bacteriocins.

5.METHODOLOGY TO BE ADOPTED

5.1 Isolation of bacteria from different sources

Samples will be collected from different sources, including soil (garden), food waste, industrial waste and polluted river water. Isolation of bacteria will be done in general purpose media using streak plate, spread plate and pour plate techniques **(eds Tiwari et al. 2009**). The colonies will be restreaked and transferred on agar slants for further use.

5.2 Screening of the isolates for the production of bacteriocin

The bacteriocinogenic action of the isolates will be monitored against several gram positive and gram-negative strains to check the inhibitory spectrum by spot overlay method and agar-well diffusion assay **(Geis et al. 1983).**

5.3 Identification and characterization of the isolates

The bacteriocin producing isolates will be identified as per Bergey's Manual of Systemic Bacteriology **(eds Krieg & Holt 1984)**.

5.4 Extraction of bacteriocin and determination its activity

5.4.1 Preparation of crude bacteriocins

After identification of the bacteriocin producing strains, the potential isolates will be grown in broth media. After processing the cells, cell free supernatant will be collected to examine for bacteriocin activity by agar-well diffusion method **(Geis et al. 1983)**.The crude bacteriocin (cell free supernatant) will be further subjected to ammonium sulphate precipitation.

5.4.2 Ammonium sulphate precipitation technique

The cell-free supernatant will be used as starting material for protein precipitation. Ammonium sulphate will be gradually added to different concentration and the precipitate will be collected (at every step) by centrifugation and re-dissolved in nutrient broth (pH 7.0) and assayed **(Harris 1989)**. The bacteriocin activity will be recorded in supernatant as well as in the precipitate at different salt concentration to get the right ammonium salt concentration for partial purification.

5.4.3 Bacteriocin activity

Bacteriocin activity will be determined by agar dilution assay after partial purification of bacteriocin **(Barefoot & Klaenhammer 1983)**.

5.5 Study the spectrum activity of bacteriocin against standard pathogenic bacterial strains

The spectrum activity of extracted bacteriocin will be examined by agar-well diffusion method against standard gram positive and gram negative pathogenic bacterial strains **(Geis et al. 1983)**.

5.6 Purification of bacteriocin

After salt precipitation further purification will be done by gel filtration and ion exchange chromatography using Sehadex G-100 and DEAE cellulose respectively.

5.6.1 Molecular weight determination

The purity check and molecular weight will be obtained by SDS-PAGE. The electrophoresis will be completed utilizing a polyacrylamide gel in Tris-glycine support framework.

5.7 Characterization of bacteriocin

5.7.1 Effect of temperature on bacteriocin activity

In order to test the heat resistance, the purified bacteriocin preparation will be heated for 10 minutes at different temperature **(Larsen et al. 1993)**. The bacteriocin activity will be detected against several gram positive and gram negative organisms.

5.7.2 Effect of pH on bacteriocin activity

Sensitivity of the purified bacteriocin at different pH will be studied as described by **Karaoglu et al. (2003).** The activity of each of the samples will be measured against the indicator organism by agar diffusion method **(Geis et al. 1983)**.

5.7.3 Effect of proteolytic and lipolytic enzymes on bacteriocin activity

The effect of proteolytic and lipolytic enzymes on bacteriocin will be examined by the treatment with protease and lipase **(Nakamura et al. 1983).**

5.7.4 Effect of surfactants, organic solvents and metal ions on bacteriocin activity

The effect of surfactants on the activity of bacteriocins will be studied by adding SDS, Tween 20 and Tween 80. The effect of metal salts on bacteriocin will be examined by adding inorganic compounds using fixed concentration and at fixed volume of the purified bacteriocin preparation **(Muriana & Klaenhammer 1991; Adinarayana et al. 2003)**.

6. RESEARCH PLAN

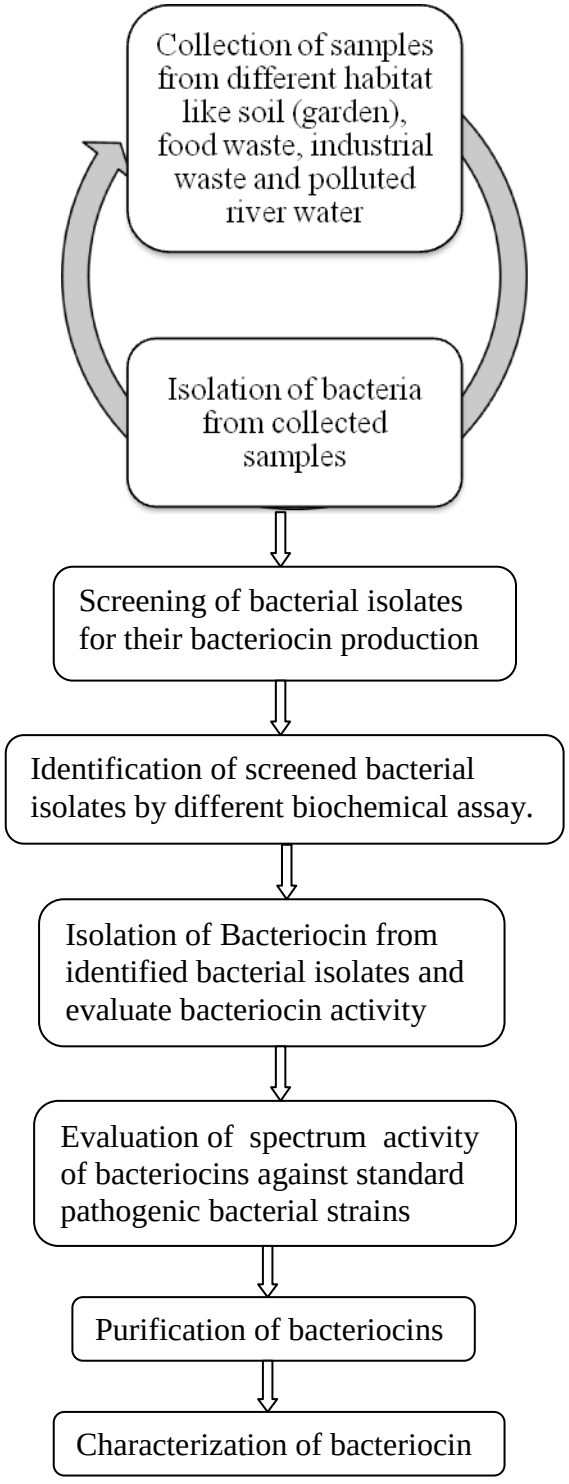
Collection of samples from different habitat like soil (garden), food waste, industrial waste and polluted river water
Isolation of bacteria from collected samples
Screening of bacterial isolates for their bacteriocin production
Identification of screened bacterial isolates by different biochemical assay.
Isolation of Bacteriocin from identified bacterial isolates and evaluate bacteriocin activity
Evaluation of spectrum activity of bacteriocins against standard pathogenic bacterial strains
Purification of bacteriocins
Characterization of bacteriocin

Figure 5 : The concise research plan for the study undertaken.

7. PROPOSED/EXPECTED OUTCOME OF THE RESEARCH

Due to increase in the antibiotic resistant pathogens, modern medicine is facing a huge problem for the treatment of various diseases. Bacteriocins offer an advantage over antibiotics due to their less toxicity, highly target specific nature and being effective at low concentrations.The only limitation in their use is the narrow spectrum mechanism of action.

Keeping in view the importance of bacteriocins in medical as well as in the food industry, the current study will plan with the aim to discover and characterize the broad spectrum bacteriocins. Initially, the study will be focused on finding the bacteriocin producing strains from different habitats, followed by isolation of potential isolates from whom the bacteriocin will be extracted , purified and characterized. After that a comparative study on the action of bacteriocin on standard gram positive and gram negative markers will enable to identify the broad spectrum bacteriocins. These are the much sought antimicrobial peptides for alternative treatments against antibiotics. This could be a great accent to the antimicrobial therapy field.

Also, bacteriocins could be used for food preservation as probiotics because these are natural peptides having antimicrobial activity and will not cause any undesirable or adverse effects on food. These bacteriocins can also be used for veterinary purpose and can kill the infection in cows and buffalos or to remove the infection that could be occurring in milk.

8. SIGNIFICANCE

Antibiotic resistance has the potential of turning common infections like fever, sore throat into diseases with high morbidity and mortality. Besides antibiotics side-effects include allergic reactions (Penicillin), electrocardiograph change and impaired renal functions. Also, other common side effects are diarrhoea, nausea vomiting , opportunistic infections of the mouth, digestive tract and vagina etc. In contrast to this, bacteriocins are biologically active proteins formed as primary metabolites of bacteria, easily hydrolyzed in the gut and have no side effects. Bacteriocins are mostly narrow spectrum and till today this restricts the use in antimicrobial chemotherapy. But broad spectrum bacteriocins can be an answer to the dreaded problem of antibiotic resistance. The proposed study can significantly contribute to the availability as an alternative to antibiotics for medical, veterinary and food industry.

9. REFERENCES

1. Adinarayana, K, Ellaiah, P & Prasad, DS 2003, 'Purification and characterization of thermostable alkaline protease from a newly isolated Bacillus subtilis PE-11, *AAPS Pharmacuticle Science Technology*, vol. 4, no. 4, pp. 56-64.

2. Aly, E & Abo, A 2007, 'Characterization of a bacteriocin like inhibitory substance produced by *Lactobacillus plantarum* isolated from Egyptian home-made yogurt', *Science Asia*, vol. 33, pp. 313-319.

3. Aymerich, T, Garriga, M, Ylla, J, Vallier, J, Monfort, JM & Hugas, M 2000a, 'Application of enterocins as biopreservatives against Listeria innocua in meat products', *Journal of Food Protection*, vol. 63, pp. 721-726.

4. Aymerich, T, Artigas, MG, Garriga, M, Monfort, JM & Hugas, M 2000b, 'Effect of sausage ingredients and additives on the production of enterocin A and B by Enterococcus faecium CTC492. Optimization of in vitro production and anti-listerial effect in dry fermented sausages', *Journal of Applied Microbiology*, vol. 88, pp. 686-694.

5. Barefoot, SF & Klaenhammer, TR 1983, 'Detection and activity of lactacin B, a bacteriocin produced by *Lactobacillus acidophilus*', *Applied and Environmental Microbiology*, vol. 45, no. 6, pp. 1808-1815.

6. Bianchi, AA & Baneyx, F 1999, 'Stress responses as a tool to detect and characterize the mode of action of antimicrobial agents', *Applied and Environmental Microbiology*, vol. 65, no. 11, pp. 5023-7.

7. Campo, R, Tenorio, C, Jime, R, Rubio, C, Baquero, F & Torres, C 2001, 'Bacteriocin Production in Vancomycin-Resistant and Vancomycin-Susceptible Enterococcus Isolates of Different Origins', *Anticicrobial agents and chemotherapy*, vol. 45 no. 3, pp. 905-912.

8. Cleveland, J, Montville, TJ, Nes, IF & Chikindas ML 2001, ' Bacteriocins: Safe, natural antimicrobials for food preservations', *International Journal of Food Microbiology*, vol. 71, no. 1, pp. 1-20.

9. Cotter, PD, Ross, RP & Hill, C 2012, 'Bacteriocins - a viable alternative to antibiotics?', *Nature Reviews Microbiology*, vol. 11, no. 2, pp. 95-105.

10. Dasarathan, R, Shivasanmugam, K & Gurunathan, J 2012, 'Isolation and partial characterization of a new bacteriocin from Bacillus pumillus DR2 isolated from sea water', *Journal of Microbiology*, vol. 1, no. 1, pp. 33-41.

11. De Vuyst, L & Vandamme, EJ 1994, 'Nisin, lantibiotic produced by *Lactococcus lactis* subsp. lactis: properties, biosynthesis, fermentation and applications' in *Bacteriocin of lactic acid bacteria. Microbiology, genetics and applications*, eds L de Vuyst & EJ Vandamme, Blackie Academic & Professional, London, pp. 151-221.

12. Dutta, S, Chakraborty, P & Ray, B 1999, 'Production, purification and characterization of enterocin Cal 1, a bacteriocin of Enterococcus faecium Cal 1, isolated from goat meat', *Indian Journal of Microbiology*, vol. 39, pp. 15-22.

13. Faith, MJ, Skvirsky, R, Gilson, L, Mahanty, HK & Kolter, R 1992, 'The secretion of colicin V' in *Bacteriocins, microcins, and lantibiotics*, eds R James, C Lazdunski & F Pattus, Springer-Verlag, New York, pp. 331-348.

14. Fimland, G, Eijsink, VGH & Nissen-Meyer, J 2002, 'Comparative studies of immunity proteins of pediocin-like bacteriocins', *Microbiology*, vol. 148, pp. 3661-3670.

15. Fredericq, P 1957, 'Colicins', *Annual Review of Microbiology*, vol. 11, pp. 7-22.

16. Fujimura, S & Nakamura, T 1979, 'Sanguicin, a Bacteriocin of Oral *Streptococcus* sanguis', *Antimicrobial agents and chemotherapy*, vol. 16, no. 3, pp. 262-265.

17. Galvez, A, Lopez, RL, Abriouel, H, Valdivia, E & Benomar, N 2008, 'Application of bacteriocins in the control of food borne pathogenic and spoilage bacteria', *Critical Reviews in Biotechnology*, vol. 28, no. 2, pp. 125-152.

18. Ganzle, MG 2004, 'Reutericyclin: biological activity, mode of action and potential application', *Applied Microbiology and Biotechnology*, vol. 64, pp. 326-332.

19. Geis, A, Singh, J & Teuber, M 1983, 'Potential of lactic streptococci to produce bacteriocin', *Applied and Environmental Microbiology*, vol. 45, pp. 205-211.

20. Ghrairi, T, Chaftar, N & Hani, K 2012, 'Bacteriocins: recent advances and opportunities' in *Progress in Food Preservation*, eds R Bhat, A Karim Alias & G Paliyath, Wiley-Blackwell, Oxford, UK, pp. 485-511.

21. Gillor, O, Nigro, LM & Riley, MA 2005, 'Genetically engineered bacteriocins and their potential as the next generation of antimicrobials', *Current Pharmaceutical Design*, vol. 11, no. 8, pp. 1-9.

22. Gratia, A 1925, 'Sur un remarquable exemple d'antagonisme entre deux souches de colibacille', *Comptes rendus des séances de la Société de biologie et de ses filiales*, vol. 93, pp. 1040-1041.

23. Guani-Guerra, E, Santos-Mendoza, T, Lugo-Reyes, SO & Teran, LM 2010, 'Antimicrobial peptides: general overview and clinical implications in human health and disease', *Clinical Immunology*, vol. 135, no. 1, pp. 1-11.

24. Harris, ELV, Angal, S & Dunn, MN 1989, 'Initial Protein purification Methods' in *A Practical Approach*, eds ELV Harris & S Angal, IRL Press, Oxford, New York, pp. 1-13.

25. Hartnett, DJ, Vaughan, A & Van Sindereu, D 2002, 'Antimicrobial producing lactic acid bacteria isolated from raw barley and sorghum', *Journal of the Institute of Brewing* ,vol. 108, no. 2, pp. 169-177.

26. Herich, R & Levkut, M 2002, 'Lactic acid bacteria, probiotics and immune system', *Veterinary Medicine - Czech*, vol. 47, no. 6, pp. 169-180.

27. Jabeen, N, Gul, H, Subhan, S, Hussain, M, Azaz, M & Rasool, S 2009, 'Biophysiochemical characterization of bacteriocin from indigenously isolated *Agrobacterium radiobacter* NA 6', *Pakistan Journal of Biotechnology*, vol. 6, pp. 3227-3237.

28. Jack, RW, Tagg, JR & Ray, B 1995, 'Bacteriocins of gram-positive bacteria', *Microbiology Reviews*, vol. 59, pp. 171-200.

29. Jacob, F, Lwaff, A, Siminonvich, A & Wallman, E 1953, 'Definition de qualques terms relatifs à la Lysogenie', *Annales de Institut Pasteur*, vol. 84, pp. 222-224.

30. Jin, T & Zhang, H 2008, 'Biodegradable polylactic acid polymer with nisin for use in antimicrobial food packaging', *Journal of food Science*, vol. 73, no. 3, pp. 127-134.

31. Karaoglu, A, Aydin, F, Kilic, S & Kilic, A 2003, 'Antimicorbial activity and characteristics of bacteriocins produced by vaginal lactobacilli', *Turkish Journal of Medical Sciences*, vol. 33, pp. 7-13.

32. Klaenhammer, TR 1993, 'Genetics of bacteriocin produced by lactic acid bacteria', *FEMS Microbiology Reviews*, vol. 12, pp. 39-86.

33. Khan, H, Flint, S & Yu, PL 2010, 'Enterocins in food preservation', *International Journal of Food Microbiology*, vol. 141, no. 1-2, pp. 1-10.

34. Krieg, NR & Holt, JC (eds) 1984, *Bergey's Manual of Systematic Bacteriology*, 1st edn, vol. 1, Williams and Wilkins, Baltimore.

35. Lancaster , LE, Wintermeyer, W & Rodnina, MV 2007, 'Colicins and their potential in cancer treatment', *Blood Cells Molecules and Diseases*, vol. 38, pp. 15-18.

36. Larsen, AG, Vogensen, FK & Josephen, J 1993, 'Antimicrobial activity of Lactic acid bacteria isolated from sour doughs: purification and characterization of bavaricin A, a bacteriocin produced by Lactobacillus bavaricus M1401', *Journal of Applied Bacteriology*, vol. 75, pp. 113-122.

37. Line, JE, Svetoch, EA, Eruslanov, BV, Perelygin, VV, Mitsevich, EV & Mitsevich, IP 2008, 'Isolation and Purification of enterocin E-760 with broad antimicrobial activity against gram-positive and gram-negative bacteria', *Antimicrobial Agents Chemotherapy*, vol. 52, pp. 1094-1100.

38. Madigan, M & Martinko, J 2005, *Brock Biology of Microorganisms*, 11th edn, Prentice Hall, Indiana.

39. Mezaini, A, Chihib, NE, Bouras, AD, Nedjar-Arroume, N & Hornez, JP 2009, 'Antibacterial activity of some lactic acid bacteria isolated from an Algerian dairy product', *Journal of Environmental and Public Health*, vol. 2009, no. 2009.

40. Motta, AS & Brandelli, A 2003, 'Influence of growth conditions on bacteriocin production by Brevibacterium linens', *Applied Microbiology and Biotechnology*, vol. 62, no. 2-3, pp. 163-167.

41. Muriana, PM & Klaenhammer, TR 1991, 'Purification and partial characterization of Lactacin F, a bacteriocin produced by *Lactobacillus acidophilus* 11088', *Applied and Environmental Microbiology*, vol. 57, pp. 114-121.

42. Nakamura, T, Yamazaki, N, Taniguchi, H, & Fujimura, S 1983, 'Production, purification and properties of a bacteriocin from *Staphylococcus aureus* isolated from saliva, *Infection and Immunity*, vol. 39, no. 2, pp. 609-614.

43. Nes, IF, Diep, DB, Halvarstein, LS, Brurberg, MB, Eijsink, V & Holo, H 1996, 'Biosynthesis of bacteriocins in lactic acid bacteria', *Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology*, vol. 70, pp. 113-128.

44. Ohno, S, Iwashita, Y, Suzuki, K & Imahoni , K 1977, 'Purification and Charecterization of active component and active fragment of Colicin E3', *The Journal of Biochemistry*, vol. 82, pp. 1045-1053.

45. Pattnaik, P, Kaushika, JK, Grover, Sn & Batish, VK 2001, 'Purifciation and characterization of a bacteriocin-like compound (Lichenin) produced anaerobically by Bacillus licheniformis isolated from water buffalo', *Journal of Applied Microbiology*, vol. 91, pp. 636-645.

46. Pal, V, Jamuna, M & Jeevaratnam, K 2005, 'Isolation and Characterization of Bacteriocin Producing Lactic acid Bacteria from a South Indian Special Dosa (appam) batter', *Journal of culture collections*, vol. 4, pp. 53-60.

47. Reveis, P 1965 , 'The bacteriocin', *Bacterial Reviews*, vol. 29, pp. 24-45.

48. Riley, MA & Wertz, JE 2002, 'Bacteriocin diversity: ecological and evolutionary perspectives', *Biochimie*, vol. 84, pp. 357-364.

49. Saleem, F, Ahmed, S, Yaqoob, Z & Rasool S 2009, 'Comperative study of two bacteriocins produced by representative indigenous soil bacteria' *Pakistan Journal of Pharmacutical Sciences*, vol. 22, no. 3, pp. 252-258.

50. Sharma N, Kappor, G & Neopaney, B 2006, 'Characterization of a new bacteriocin produced from a novel isolated strain of Bacillus lentus NG121', *Antonie Van Leeuwenhoek*, vol. 89, pp. 337-343.

51. Sharma, N, Kapoor, R, Gautam N & Kumari R 2011, 'Purification and Characterization of Bacteriocin Produced by Bacillus subtilis R75 Isolated from Fermented Chunks of Mung Bean (*Phaseolus radiatus*)' *Food Technology Biotechnology*, vol. 49, no. 2, pp. 169-176.

52. Tiwari, RP, Hoondal, GS & Tewari, R (eds) 2009, *Laboratory Techniques in Microbiology and Biotechnology*, Abhishek Publications, Chandigarh, India.

53. Van Heel, AJ, Montalban-Lopez, M & Kuipers, OP 2011, 'Evaluating the feasibility of lantibiotics as an alternative therapy against bacterial infections in humans', *Expert Opinion on Drug Metabolism & Toxicology*, vol. 7, pp. 675-680.

54. Yuping, L & Richard, LG 2009, 'AMPed Up immunity: how antimicrobial peptides have multiple roles in immune defense', *Trends Immunology*, vol. 30, no. 3, pp. 131-141.

55. Walker, ES & Levy, F 2001, 'Genetic trends in population evolving antibiotic resistance', *Evolution*, vol. 55, pp. 1110-1122.